SPECIAL GUEST EDITOR SECTION

# Triglyceride-Lowering Response to Plant Sterol and Stanol Consumption

**TODD C. RIDEOUT and CHRISTOPHER P.F. MARINANGELI**
University at Buffalo, School of Public Health and Health Professions, Department of Exercise and Nutrition Sciences, Buffalo, NY 14214
**SCOTT V. HARDING**
King's College London, School of Medicine, Diabetes and Nutritional Sciences Division, London SE1 9NH, United Kingdom

**Phytosterols (PS) have long been recognized for their cholesterol-lowering action, however, recent work has highlighted triglyceride (TG)-lowering responses to PS that may have been overlooked in previous human interventions and mechanistic animal model studies. This review assesses the current state of knowledge regarding the effect of dietary PS supplementation on blood TG concentrations by examining the average therapeutic response, potential mechanisms, and metabolic and genetic factors that may contribute to inter-individual variability. Data from human intervention trials demonstrates that, compared to baseline concentrations, PS supplementation results in a variable TG-lowering response ranging from 0.8 to 28%. It is evident that hypertriglyceridemic individuals (>1.7 mmol/L) have a greater TG-lowering response to PS (11–28%) than subjects with normal plasma TG concentrations (0.8–7%). Although a genetic basis for the variable TG-lowering effects of PS is probable, there are only limited studies to draw on. The available data suggest that polymorphisms in the apolipoprotein E (apoE) gene may affect responsiveness, with PS-induced reductions in TG more readily evident in apoE2 than apoE3 or E4 subjects. Although only a minimal number of animal model studies have been conducted to specifically examine the mechanisms whereby PS may reduce blood TG concentrations, it appears that there may be multiple mechanisms involved including interruption of intestinal fatty acid absorption and modulation of hepatic lipogenesis and very low density lipoprotein packaging and secretion. In summary, the available data suggest that PS may be an effective therapy to lower blood TG, particularly in hypertriglyceridemic individuals. However, before PS can be widely recommended as a TG-lowering therapy, studies that are specifically powered and designed to fully access therapeutic responses and the mechanisms involved are required.**

Guest edited as a special report on "Safety, Health, and Methodological Aspects of Plant Sterols and Stanols" by Peter Jones and Dylan MacKay.

Corresponding author's e-mail: rideout@buffalo.edu

DOI: 10.5740/jaoacint.SGERideout

Since discovery of the association between elevated blood cholesterol and increased cardiovascular disease (CVD) risk, early animal (1) and epidemiological investigations (2), diet-based and pharmacological cholesterol-lowering therapies have become integral components of primary and secondary CVD prevention programs. Although these therapies have largely reduced the prevalence of high low-density lipoprotein cholesterol (LDL-C) among Americans, close to 33% of U.S. adults still have elevated LDL-C, and there is concern that high-risk individuals often fail to meet their LDL-C target goals.

Phytosterols (PS), plant-based sterols that structurally resemble cholesterol, are arguably the best-defined nutraceutical approach to reduce blood cholesterol concentrations by interfering with intestinal cholesterol absorption. PS have a proven track record as 'natural' cholesterol-lowering agents with consistent LDL-C reductions in the range of 8–16% in numerous well-controlled clinical interventions (3) and preclinical studies that have defined the molecular mechanisms involved (4–6). Although PS are highly regarded as effective for reducing circulating total cholesterol and LDL-C, they were traditionally believed to have no effect on triglyceride (TG) concentrations, an important independent CVD risk factor. However, recent animal and human studies have highlighted a potential role for PS in regulating blood TG concentrations [Table 1 (7–19) and Table 2 (20–40)]. That the TG-lowering properties of PS are just now surfacing may seem unexpected given that their health benefits have been actively researched in controlled human studies since the 1950s. However, a close assessment of previous clinical PS interventions reveals TG-lowering responses that may have been overlooked for a variety of reasons. First, the lipid hypothesis placed such a major emphasis on cholesterol as the major CVD risk factor that PS interventions were specifically designed and statistically powered to detect movement in the primary endpoint of LDL-C, not other lipid risk factors. Furthermore, recent work suggests that the TG lowering responses of PS are most clearly observed in hypertriglyceridemic individuals; however, the vast majority of PS interventions were designed with hypercholesterolemia as the main study inclusion criteria. Finally, the TG-lowering action of PS may have been difficult to discern as metabolic and genetic factors may contribute to a relatively variable response compared with the more consistent reductions observed in circulating cholesterol levels.

This review will provide a thorough assessment of the effects of PS on TG metabolism with discussion of the TG-lowering effects reported in previous clinical interventions and what is known regarding the potential molecular mechanisms that may

**Table 1. Selected clinical studies reporting changes in plasma triglyceride concentrations in response to phytosterol/phytostanol supplementation**

| Study | *n* | | Duration | Design | Dose | Vehicle | Baseline TG, mmol/L | Effect on TG level, % change from baseline |
|---|---|---|---|---|---|---|---|---|
| Maki et al. (7) | 28<br>7 M[a]<br>21 F[b] | Primary hypercholesterolemia | 6 weeks | Randomized, single-blinded, placebo-controlled, crossover<br>NCEP TLC[c] weight maintenance diet | 1.8 g/day<br>(81% PS & 19% PSA) | Capsule | 1.41 | versus Base: ↓4.4%<br>versus Control:↓9.1% |
| Sialvera et al. (8) | 101<br>60 M<br>48 F | Metabolic syndrome | 2 months | Randomized, single-blinded, placebo-controlled, parallel-arm<br>Western-type diet | 4 g/day PS | Yogurt beverage | 2.20 | versus Base: ↓24%<br>versus Control: ↓12.5% |
| Sanchez-Muniz et al. (9) | 217 | Hypercholesterolemic, stratified by carrier of the Apo E2 (E2E2), E3 (E3E3) and E4 (E3E4, E4E4) alleles | 5 weeks | Randomized, double-blinded, placebo-controlled parallel-arm<br>NCEP Step 1 diet | 1.1–2.2 g/day PS | Margarine | E2: 1.66<br>E3: 1.33<br>E4: 1.69 | E2<br>versus Control: ↓15.5%<br>E3<br>versus Control: ns[d]<br>E4<br>versus Control: ns |
| Theuwissen et al. (10) | 28<br>16 M<br>12 F | Elevated triglyceride levels | 3 weeks | Randomized, double-blinded, placebo-controlled parallel-arm<br>Habitual diet | 2.5 g/day PSA[e] | Margarine | 2.63 | versus Base: ↓11%<br>(subjects with baseline TG > 2.3 mmol/L) |
| Plat et al. (11) | 18 | Dyslipidemic metabolic syndrome | 8 weeks | Randomized, double-blinded, placebo-controlled, parallel-arm | 2.0 g/day PSA | Yogurt beverage | 2.21 | versus Base: ↓28% |
| | 75 | Normolipidemic | 8 weeks | Randomized, double-blinded, placebo-controlled, parallel-arm | 3.8–4.1 g/day PSA | Margarine and shortenings | 1.02 | ns |
| Rideout et al. (12) | 33<br>15 M<br>18 F | Normolipidemic | 28 days | Randomized, placebo-controlled, crossover<br>Controlled feeding trial (50% CHO,[f] 35% fat, 15% protein) | 1.95 g/day PS | Low fat soy beverage | 1.65 | ns |
| | 23<br>10 M<br>13 F | Hypercholesterolemic | 28 days | Randomized, placebo-controlled, crossover<br>Controlled feeding trial (50% CHO, 35% fat, 15% protein) | 1.95 g/day PS | Moderate fat soy beverage | 2.06 | versus Control: ↓9.4% |
| Plat et al. (13) | 36<br>23 M<br>13 F | Metabolic syndrome | 8 weeks | Randomized, placebo-controlled parallel-arm | 2.0 g/day PSA | Yogurt beverage | 2.21 | versus Base:↓15.9%<br>versus Control:↓44.5% |
| Clifton et al. (14) | 151<br>77M<br>74F | Hypercholesterolemic | 6 weeks | Randomized, double-blinded, placebo-controlled, parallel-arm<br>Habitual diet | Weeks 0–3: 1.6 g/day PS<br>Weeks 3–6: 3.0 g/day PS | Margarine | 1.92 | Weeks 0–3<br>versus Base:↓12.5%<br>versus Control: ↓17.4%<br>Weeks: 3–6<br>versus Base:↓12.5%<br>versus Control: ↓21.5% |
| Plana et al. (15) | 83 | Hypercholesterolemic | 6 weeks | Multicenter, randomized, double-blind, placebo-controlled, parallel-arm | 1.6 g/day PS | Yogurt beverage | 1.55 | versus Base: ↓7%<br>versus Control: ↓14% |

**Table 1. (*continued*)**

| Study | *n* | | Duration | Design | Dose | Vehicle | Baseline TG, mmol/L | Effect on TG level, % change from baseline |
|---|---|---|---|---|---|---|---|---|
| Judd et al. (16) | 53<br>26 M<br>27 F | Normocholesterolemic and mildly hypercholesterolemic | 3 weeks | Randomized, placebo-controlled, double-blind, crossover | 3.6 g/day PS | Salad dressing | 1.46 | versus Control: ↓7.3% |
| Davidson et al. (17) | 84<br>46 M<br>38 F | Healthy | 8 weeks | Randomized, double-blind, placebo-controlled, parallel-arm<br><br>Habitual diet | 3.0 g/day PS<br>6.0 g/day PS<br>9.0 g/day PS | Reduced fat spread and/or salad dressing | 1.20<br>1.18<br>1.47 | 3.0 g/day, versus Base: ↓13.3%<br>6.0 g/day, versus Base: ns<br>9.0 g/day, versus Base: ns |
| Jones et al. (18) | 15 M | Hypercholesterolemic | 21 days | Randomized, double-blind, placebo-controlled, crossover<br><br>Controlled feeding trial (50% CHO, 35% fat, 15% protein) | 1.84 g/day PS<br>1.84 g/day PSA | Margarine | 2.52<br>2.39 | PS<br>versus Base:↓18.9%<br>versus Control: ↓1.0%<br>PSA<br>versus Base:↓17.4%<br>versus Control: ↓0.9% |
| Shaghaghi et al. (19) | 47<br>25 M<br>22 F | Mild to moderately hypercholesterolemic | 29 days | Randomized, placebo-controlled, crossover<br>Habitual diet | 2.0 g/day water dispersible PS | Yogurt | 1.68 | versus Base: ↓0.8%<br>versus Control: ↓13.9% |

[a] M = Male.

[b] F = Female.

[c] NCEP TLC = National Cholesterol Education Program.

[d] ns = Not significant.

[e] PSA = Plant stanols and PS = Plant sterols.

[f] CHO = Carbohydrate.

**Table 2. Selected preclinical studies reporting plasma and/or tissue triglyceride responses following phytosterol/phytostanol supplementation**

| Author | Model | Diet and Design | Result | Notes |
|---|---|---|---|---|
| | | Hamster model | | |
| Rideout et al. (20) | Male Syrian golden | Semisynthetic 'Western' diet 2% PS supplementation (Reducol)<br>6 weeks feeding | ↓ blood TG (49%)<br>Shift in hepatic FA (↑ 16:0; ↓16:1 and 18:1<br>↓ de novo lipogenesis (44%)<br>↓ intestinal SREBP1c, hepatic PPARα, and FAS mRNA[b] | Comparable TG reductions to ezetimibe supplementation<br>Reductions in blood and hepatic cholesterol levels also observed |
| Ntanios et al. (21) | Male and female Syrian golden | Semisynthetic diet supplemented with PS (0.5 or 1%) from tall oil or soybean oil<br>Approximately 13 weeks feeding | ↓ blood TG in males fed 1% soybean oil-PS (no % given) | No effect in female hamsters |
| Ntanios et al. (22) | Male $F_1B$ Syrian golden | Semisynthetic diet enriched with graded doses of phytosterol ester (0.24–2.84%). | ↓ blood TG in animals fed >0.96% phytosterol ester (0.96, 1.92, 2.84%) | |
| Vanstone et al. (23) | Male Syrian golden | Semisynthetic diet; tall oil or soybean oil derived PS supplemented in diet or subcutaneously injected (matched to 5 mg/kg BW/day)<br>Approximately 9 weeks feeding | ↓ blood TG (-42%) in animals injected with soybean oil derived PS | |
| Jain et al. (24) | Male Syrian golden | Semisynthetic, cholesterol-enriched diet with sitostanol (0.5%) | ↓ blood TG (–22%) in sitostanol group compared with control | |
| Liang et al. (25) | Male Syrian golden | Semisynthetic, cholesterol-enriched diet supplemented with sitosterol or stigmasterol (0.1%) | ↓ blood TG (–28%) in both groups | ↓ in intestinal mRNA expression of microsomal triglyceride transfer protein in both groups |
| Ebine et al. (26) | Male Syrian golden | Semisynthetic, cholesterol-enriched diet supplemented with 0.7 and 1.4% disodium ascorbyl phytostanyl phosphate[a] | ↓ blood TG (–45%) in 1.4% supplemented group | |
| | | Mouse model | | |
| Rideout et al. (27) | Male C57BL/6J | Semisynthetic 'Western' diet 2% PS supplementation (Reducol)<br>6 weeks feeding | ↓ blood TG (–28%)<br>↓ hepatic TG (30%)<br>↑ fecal 16:0 and 18:0 excretion<br>↑ hepatic SREBP1c and FAS mRNA; ↓ intestinal PPARα mRNA<br>↑ de novo lipogenesis (23%) | Blood total cholesterol not altered<br>No change in a host of intestinal FA absorption and metabolism gene expression |
| Volger et al. (28) | Female apolipoprotein E*3-Leiden transgenic | Semisynthetic containing 0.25% cholesterol and 0.0, 0.25, 0.5, 0.75, or 1.0% plant stanols<br>8 weeks feeding | ↓ hepatic TG (–38%) in the 1% dietary stanol group | No effect on serum TG<br>No effect on hepatic VLDL-TG secretion |
| Plosch et al. (29) | Male C57BL/6J | Semisynthetic cholesterol supplemented diet with 0.5% plant sterols or stanols<br>4 weeks feeding | ↓ hepatic TG in both sterol and stanol groups | No change in plasma TG concentrations |
| Brufau et al. (30) | Male C57BL/6J | Semisynthetic cholesterol supplemented diet with 1, 2, 4, or 8% plant sterols<br>2 weeks feeding | ↓ blood TG (–26%) in 4% supplemented group<br>↑ hepatic TG (59%) in the 2% supplemented group | |
| Looije et al. (31) | Male C57BL/6J | Low fat semisynthetic diet supplemented with 2% FM-VP4[a] | ↓ blood TG (% reduction not specified) | |

**Table 2. (*continued*)**

| Author | Model | Diet and Design | Result | Notes |
|---|---|---|---|---|
| Lukic et al. (32) | Male apoE knockout | Cholesterol-supplemented chow diet with 0.1, 0.5, 1.0, and 2.0% FM-VP4[a]<br><br>12 weeks feeding | ↓ blood TG in 2% supplemented group at 4 and 8 weeks | |
| Schonewille et al. (33) | Male C57BL/6J | Semisynthetic high fat/ cholesterol diet supplemented with 3.1% plant sterols or stanol esters | ↓ blood TG<br><br>No effect on hepatic TG levels | Down regulation of LXR target genes in liver including ATP binding cassette A1, G5, and G8, and sterol regulatory element binding protein 1c [c] |
| | | Rat model | | |
| Matasuoka et al. (34) | Male Sprague-Dawley rats | Semisynthetic diet supplemented with 0.5% free PS (FPS) or free PS egg yolk lipoprotein complex (PSY)<br><br>3 weeks feeding | ↓ hepatic TG in both groups (FPS, –33%; PSY, –22%) | No change in serum TG concentrations |
| Awaisheh et al. (35) | Male Sprague-Dawley rats | Semipurified high fat/cholesterol chow; daily gavage with nonfermented milk with and without PS (5 mg/mL)<br><br>8 weeks feeding | ↓ blood TG (–16%)<br><br>↓ hepatic TG (–92%) | |
| Ikeda et al. (36) | Male Sprague-Dawley rats | Semisynthetic diet supplemented with campestenone (0.5%) | ↓ blood TG (–76%)<br><br>↓ hepatic TG (–69%)<br><br>↑ expression of β-oxidation genes<br><br>↓ hepatic SREBP1c expression | |
| Tomoyori et al. (37) | Male Sprague-Dawley rats | Semisynthetic supplemented with 0.25% PS | ↓ lymphatic transport of TG | |
| | | Pig model | | |
| Brufau et al. (38) | Female Dunkin Hartley guinea pigs | Cholesterol enriched (0.33%), isocaloric diets, chow versus semisynthetic not specified; Supplemented with three doses of PS (0, 1.27, and 2.45%);<br><br>Diets with combination pectin and PS were also examined<br>4 weeks feeding | ↑ apparent absorption of saturated FA in PS-supplemented animals including lauric (12:0) and myristic (14:0) acids<br><br>↑ hepatic incorporation of lauric (12:0) and myristic (14:0) acids in PS-supplemented animals versus animals fed saturated fat diet | |
| Brufau et al. (39) | Female Dunkin Hartley guinea pigs | | ↓fecal excretion of lauric (12:0) and myristic (14:0) acids compared with high saturated fat diet<br><br>↑ fecal excretion of arachidic (20:0) and behenic (22:0) acids compared with high saturated fat diet | |
| Brufau et al. (40) | Female Dunkin Hartley guinea pigs | | No change in plasma TG | |

[a] A semisynthetic esterified phytostanols-ascorbic acid derivative.

[b] SREBP1c = Sterol regulatory element binding protein 1c, PPARα = peroxisome proliferator receptor alpha, and FAS = fatty acid synthase.

[c] LXR = Liver x receptor and ATP = adenosine triphosphate.

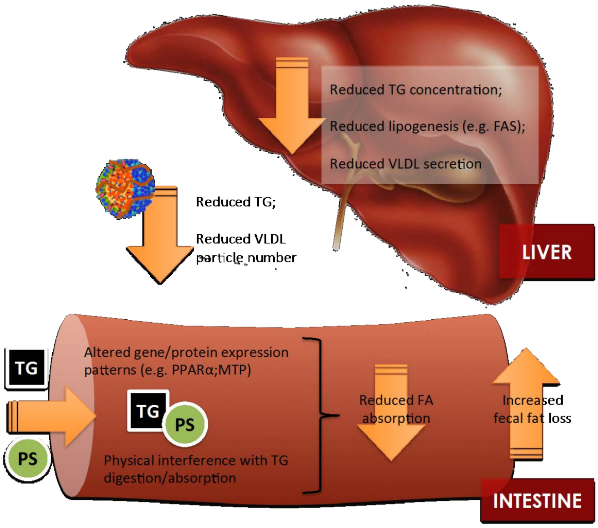


**Figure 1. Potential mechanisms involved in the triglyceride-lowering response to physterols/phytostanols.**

underlie these responses. We review the extent of our knowledge regarding the metabolic and genetic factors that are thought to influence these responses and discuss future research priorities that must be addressed to more fully evaluate PS as a potential TG-lowering therapy.

## Clinical Assessment of TG Lowering in Response to PS

A review of the clinical trial database demonstrates a TG-lowering effect of PS ranging from 0.8 to 28% compared to baseline values (7–19). The TG-lowering responses of selected studies are presented in Table 1.

A purview of this data demonstrates that, for the most part, the TG-lowering efficacy of PS increases as baseline TG levels become more prominent. For example, in studies where subjects were hypertriglyceridemic (>1.7 mmol/L), 1.6–4 g/day PS lowered circulating TG levels 11–28% (8, 10–14, 18). Conversely, in studies where baseline TG levels were <1.7 mmol/L, 1.6–4.1 g/day PS lowered TG levels by 0.8–7% (7, 11, 12, 15, 16, 19). Pooled analysis of five clinical studies showed that subjects with baseline TG concentrations of 1.0 mmol/L experienced a 1.0, 3.8, and 4.7% reduction in circulating TG levels with 2.0, 3.0. or 4.0 g/day plant stanols, respectively (40). However, across the same dosages of PS, when baseline TG values were 2.0 and 3.0 mmol/L, TG levels were decreased by approximately 1.5 and 2.0%, 5.8 and 7.8%, and 7.0 and 9.7%, respectively (41). Using data from 12 clinical trials, Demonty et al. (42) showed PS facilitated a mild decrease in circulating TG of 6.0%. However, with the exception of two subjects with mildly elevated baseline TG levels (1.73 and 1.93 mmol/L), baseline TGs were relatively normal. Therefore, the 6.0% reduction in circulating TG aligns with data demonstrated in Table 1. In the same study, when the data were stratified by baseline TG levels, PS lowered TG concentrations by 0.18 mmol/L in subjects with TG levels within the 75th percentile (1.9 mmol/L; 42). This reduction is substantially greater than the 0.0006 and 0.08 mmol/L decrease in circulating TG concentrations among subjects with baseline TGs within the 25th (0.99 mmol/L) and 50th percentile (1.36 mmol/L), respectively (42). To date, only one clinical trial, by Theuwissen et al. (10), has sought to delineate PS TG-lowering efficacy. Using subjects with baseline TG levels of at least 1.73 mmol/L, PS had no effect on circulating TG concentrations. However, among subjects with moderate-to-high baseline TG levels (>2.3 mmol/L), 2.5 g/day PS lowered TG concentrations by 11% compared with baseline. It is noted that among normotriglyceridemic subjects (1.2 mmol/L), Davidson et al. (17) observed a 13.3% decrease in TG levels with 3.0 g/day. However, no effect was demonstrated with 6.0 and 9.0 g/day PS (17). While the TG-lowering results outlined in Table 1 are promising, a more focused approach for delineating PS effects on circulating TG is required. Overall, data suggest that 2.0–4.0 g/PS/day facilitates significant reductions in circulating TG levels in humans. However, the degree of PS-induced TG lowering could be dependent on the presence and magnitude of hypertriglyceridemia.

In addition to applying hypertriglyceridemia as inclusion criteria in future clinical trials, future studies should also consider the consistency as to how the TG-lowering response is reported. Table 1 shows that the TG-lowering efficacy of PS is reported as comparisons to baseline, the control group, or both. This is problematic when comparing the effects PS on circulating TG levels between studies. For example, Jones et al. (18) reported that, compared to baseline, hypertriglyceridemic subjects receiving 1.84 g/day PS decreased circulating TG by 18.9 and 17.4%, respectively. However, compared to the control group, TG levels were modestly decreased by 1.0% (18). Shaghaghi et al. (19) demonstrated the opposite effect, where 2.0 g/day PS decreased TG relative to baseline and the control group by 0.8 and 13.9%, respectively. Large disparities in relative reductions in TG between baseline and controls values could suggest that baseline TG levels, background diets, or other lifestyle factors are imposing a substantial effect on the TG response. Perhaps consistent reporting of absolute TG reductions would address the abovementioned discrepancies in data that support the effects of PS on elevated TG concentrations.

## Preclinical Assessment of TG-Lowering in Response to PS

Several animal species, namely, hamsters, mouse, and rat models, demonstrate fairly consistent reductions in both blood and hepatic TG concentrations following dietary PS incorporation (Table 2). It is of interest to note that these TG reductions are evident despite drastic differences in study design, including diverse animal models with distinct lipid metabolism and different background diets (chow versus semisynthetic) with variable types of fat and wide-ranging sources and supplementation levels of PS. With a few exceptions, most of these studies were designed to specifically examine cholesterol-lowering responses and associated mechanisms; however, results of these studies do shed light on potential pathways by which PS may directly or indirectly modulate TG metabolism (Figure 1).

There are multiple lines of evidence to suggest that at least part of the TG-lowering response to PS is related to alterations in TG and/or fatty acid (FA) metabolism within the intestine. In what we believe to be one of the first studies explicitly designed to examine the TG-lowering mechanisms of PS, we fed male

C57BL/6J mice a semisynthetic 'Western' diet supplemented with 2% PS (Reducol) for 6 weeks. Wild type C57BL/6J mice are a unique model as they exhibit reductions in blood and hepatic TG following PS supplementation but are considered nonresponders to the cholesterol-lowering action of PS due to their high cholesterol synthetic capacity (43). Compared with unsupplemented animals, PS-fed mice exhibited reductions in plasma (28%) and hepatic (30%) TG concentrations. At least part of this TG lowering response was associated with changes in intestinal fat metabolism including increased fecal FA excretion, specifically fecal palmitate and stearate. Supporting a potential role of PS in reducing intestinal TG absorption, Tomoyori et al. reported that PS reduced the postprandial lymphatic transport of TG (5–7 h following a meal) in thoracic duct–cannulated Sprague-Dawley rats (37). In our mouse study, we detected no difference in the expression of a host of intestinal genes related to FA absorption and chylomicron assembly; however, PPARα mRNA expression was reduced compared with control animals (discussed below). These results may suggest a physical interference of PS on intestinal FA absorption rather than a direct effect on the expression of genes and proteins that regulate FA uptake, similar to the interference of PS with the incorporation of cholesterol into bile salt micelles. Alternatively, Liang et al. reported that male Syrian golden hamsters exhibited a 28% reduction in blood TG that was associated with reduced mRNA expression of intestinal microsomal triglyceride transfer protein (MTP) following dietary supplementation with sitosterol or stigmasterol (0.1%; 25). As MTP plays a pivotal role in the assembly and secretion of apolipoprotein B (apoB)-containing chylomicron particles, PS-mediated reductions in the expression of this gene could conceivably be linked to TG lowering and should be confirmed in future mechanistic studies. Although many studies (both animal and human) have directly examined intestinal cholesterol absorption in response to PS consumption, we are not aware of any studies that have employed stable-isotope methodology to directly measure FA absorption following PS intervention. This is a major knowledge gap in our understanding of the potential mechanisms involved in PS-mediated TG reductions and should be addressed in future animal and human studies.

As mentioned above, in a previous study designed to examine the TG lowering responses of PS, we have observed reductions in intestinal peroxisome proliferator-activated receptor alpha (PPARα) mRNA expression (27). PPARα is a nuclear receptor highly expressed within intestinal enterocytes that mediates the effects of nutrients, specifically FA, on gene expression. PPARα activation has been shown to regulate a whole host of intestinal functions including nutrient transport, fatty acid oxidation, oxidative stress, and inflammation, and its expression pattern mimics that of other critical genes involved in FA absorption (44–46). The broad scope of its regulation and constant exposure to its FA ligands makes PPARα an intriguing therapeutic target for obesity and dyslipidemia. At this point, it is difficult to say whether the observed reduction in PPARα mRNA expression is a direct effect of PS or an indirect consequence, possibly from a direct interference of PS with intestinal TG digestion and FA absorption.

There is also evidence to suggest that PS directly or indirectly influence hepatic FA and TG metabolism. The liver is central to whole-body FA and TG metabolism as a primary site for the *de novo* lipogenesis of FA, the synthesis and secretion of nascent very low density lipoprotein (VLDL) particles, and the clearance of FAs from TG-rich remnant lipoprotein particles and high density lipoprotein (HDL) species. PS supplementation is regularly associated with a reduction in hepatic TG concentrations in hamster, mouse, and rat models (Table 2). In addition to tissue TG concentrations, there is also evidence to suggest that PS may modulate the hepatic FA profile. We observed a shift in hepatic fatty acid composition toward increased saturated 16:0 (approximately 50%) and reduced monounsaturated FA (16:1, approximately 40% and 18:1, approximately 24%) in PS-supplemented hamsters compared with unsupplemented animals (20). Similarly, Brufau et al. also observed an increase in the hepatic incorporation of lauric (12:0) and myristic (14:0) acids in PS-supplemented female Dunkin Hartley guinea pigs versus animals fed a saturated fat diet (38). The implications of this apparent shift in hepatic FA toward a more saturated profile is not yet known, although it may be a secondary effect due to a reduction in intestinal TG absorption or modulation of hepatic lipogenesis and/or lipoprotein synthesis. In support of a TG-lowering mechanism of hepatic origin, a recent study reported a reduction in hepatic VLDL secretion in male C57BL/6J mice fed a high fat diet supplemented with 3.1% PS or stanol esters for 3 weeks (33). Furthermore, in a human study, Plat et al. reported a reduction in large and medium plasma VLDL particles in dyslipidemic metabolic syndrome subjects consuming 2 g of PS provided in a yogurt drink matrix (11).

We have investigated the modulation of hepatic *de novo* lipogenesis as a potential mechanism that may underlie TG reductions following PS supplementation in both mouse and hamster models (20, 27). However, the results of these studies highlight differential model-specific responses that preclude any clear consensus regarding the impact of PS on hepatic lipogenesis. We observed an increase in hepatic *de novo* lipogenesis in PS-fed C57BL6 that we interpreted to be a compensatory response to interference with intestinal FA absorption as evidenced by increased fecal FA excretion. We have recently identified a similar response in a PS-fed Zucker rat model that demonstrated an increase in the ratio of hepatic 16:0/18:2n-6, an indirect measure of hepatic de novo lipogenesis (Rideout et al., unpublished). However, in a separate study, PS fed Syrian golden hamsters exhibited a reduction in *de novo* lipogenesis that was supported by a decrease in the protein abundance of FA synthase, a rate-limiting enzyme in the lipogenic pathway. Given the similarity in the design factors between the two studies, including background diet, PS supplementation level, and stable isotope analysis, this discrepancy highlights the underlying differences in lipid metabolism between mouse and hamster species and clearly demonstrates the need for estimates of *de novo* lipogenesis as part of a mechanistic human intervention.

It is of interest to note that lipid reductions (both cholesterol and TGs) have been reported following intraperitoneal and subcutaneous PS injections in various animal models (47–49). Vanstone et al. (23) observed TG reductions in Syrian golden hamsters subcutaneously injected with PS at a dose of 5 mg/kg/body weight. These results suggest that PS may mediate blood lipid concentrations independent of their direct effects within the intestine, possibly by modulating the expression of genes that regulate hepatic TG balance.

## Factors Affecting Responsiveness

Recent work has highlighted a variety of subject specific metabolic and genetic factors that predict the magnitude and direction of the cholesterol response to PS (43, 50–52). Although less work has been done to specifically examine the heterogeneity of responses in blood TG, its range has been estimated to be between 6–20% (3). As discussed previously, the TG-lowering efficacy of PS seems to depend on the presence and magnitude of hypertriglyceridemia. This stands to reason as TG reductions in response to ezetimibe, the well-characterized intestinal cholesterol absorptive inhibitor, have also been shown to be dependent on baseline TG concentrations (53).

Although a genetic basis for the variable TG-lowering effects of PS is probable, we are only aware of two studies that have examined a potential genetic link, both with polymorphisms in the apolipoprotein E (apoE) gene. ApoE is an apolipoprotein component of lipoproteins including chlyomicrons, VLDL, LDL, and HDL and is, therefore, heavily involved in directing lipoprotein metabolism and remodeling with the plasma compartment. ApoE is polymorphic with three common alleles in the population, namely ε4, ε3, and ε2, that are thought to underlie the lipid-lowering responses to drug and diet-based therapies. Sanchez-Muniz et al. observed TG reductions in apoE2 but not in apoE3 or E4 subjects following PS intervention in hypercholesterolemic adults (8). Although not significant, Geelen et al. reported that E4 subjects tended ($P = 0.13$) to have a greater TG-lowering response compared with E3/3 subjects consuming 3.2 g of daily PS in margarine (difference of 0.08 mmol/L; 54). There is a need to conduct further studies to understand the genetic basis of responsiveness with a focus on genes that are known to modulate plasma TG concentrations and lipoprotein metabolism. Furthermore, there has yet to be any studies to examine more readily identifiable patient characteristics, such as ethnicity, age, gender, and body mass index that may underlie the TG-lowering response to PS.

## Summary

Although PS are well substantiated for their LDL-C lowering effects in hypercholesterolemic patients, the efficacy of PS as a TG-lowering therapy has only recently gained momentum within the nutrition community through a limited number of animal and human studies specifically designed to examine this response and the potential mechanisms involved. Nonetheless, clinical trial data have shown that, among subjects with hypertriglyceridemia (TG >1.73 mmol/L), 2–4 g/PS/day can facilitate a decrease in TG concentrations of ≥11%. Results from a number of different animal studies suggested that the TG-lowering mechanisms of PS may be multifactorial, including interference with FA absorption within the intestinal lumen, modulation of hepatic *de novo* lipogenesis, and a reduction in circulating medium and large VLDL particles. The effects of PS on the expression of a variety of gene and protein targets, both in the intestine and liver, suggest that there may be a molecular component to the TG-lowering response. However, to fully substantiate the utility of PS as a TG-lowering therapy, human clinical trials that are specifically powered to detect an effect of PS on TG concentrations in hypertriglyceridemic subjects are required. Furthermore, human interventions should explore a mechanistic basis for the TG-lowering response with a direct examination of FA absorption and whole-body lipogenesis in response to PS supplementation. Finally, responsiveness studies that identify both metabolic and genetic factors that determine the magnitude of PS-induced TG reductions will be critical in defining the clinical utility of PS as a TG-lowering therapy.

## References


(1) Classics in Arteriosclerosis Research: On Experimental Cholesterin Steatosis and its Significance in the Origin of Some Pathological Processes by N. Anitschkow and S. Chalatow (1913) translated by Mary Z. Pelias (1983) *Arteriosclerosis* **3**, 178–182

(2) Coronary Heart Disease in Seven Countries, Summary (1970) *Circulation* **41**, 186–195

(3) Gylling, H., Plat, J., Turley, S., Ginsberg, H.N., Ellegard, L., Jessup, W., Jones, P.J., Lutjohann, D., Maerz, W., Masana, L., Silbernagel, G., Staels, B., Boren, J., Catapano, A.L., De Backer, G., Deanfield, J., Descamps, O.S., Kovanen, P.T., Riccardi, G., Tokgozoglu, L., & Chapman, M.J. (2014) *Atherosclerosis* **232**, 346–360. http://dx.doi.org/10.1016/j.atherosclerosis.2013.11.043

(4) De Smet, E., Mensink, R.P., & Plat, J. (2012) *Mole. Nutr. Food Res.* **56**, 1058–1072. http://dx.doi.org/10.1002/mnfr.201100722

(5) Amir Shaghaghi, M., AbuMweis, S.S., & Jones, P.J. (2013) *J. Acad. Nutr. Diet.* **113**, 1494–1503. http://dx.doi.org/10.1016/j.jand.2013.07.006

(6) Ras, R.T., Geleijnse, J.M., & Trautwein, E.A. (2014) *Br. J. Nutr.* **112**, 214–219. http://dx.doi.org/10.1017/S0007114514000750

(7) Maki, K.C., Lawless, A.L., Reeves, M.S., Kelley, K.M., Dicklin, M.R., Jenks, B.H., Shneyvas, E., & Brooks, J.R. (2013) *Nutrition* **29**, 96–100. http://dx.doi.org/10.1016/j.nut.2012.05.002

(8) Sialvera, T.E., Pounis, G.D., Koutelidakis, A.E., Richter, D.J., Yfanti, G., Kapsokefalou, M., Goumas, G., Chiotinis, N., Diamantopoulos, E., & Zampelas, A. (2012) *Nutr. Metab. Cardiovasc. Dis.* **22**, 843–848. http://dx.doi.org/10.1016/j.numecd.2010.12.004

(9) Sanchez-Muniz, F.J., Maki, K.C., Schaefer, E.J., & Ordovas, J.M. (2009) *J. Nutr.* **139**, 13–19. http://dx.doi.org/10.3945/jn.108.090969

(10) Theuwissen, E., Plat, J., van der Kallen, C.J., van Greevenbroek, M.M., & Mensink, R.P. (2009) *Lipids* **44**, 1131–1140. http://dx.doi.org/10.1007/s11745-009-3367-6

(11) Plat, J., & Mensink, R.P. (2009) *Lipids* **44**, 1149–1153. http://dx.doi.org/10.1007/s11745-009-3361-z

(12) Rideout, T.C., Chan, Y.M., Harding, S.V., & Jones, P.J. (2009) *Lipids Health Dis.* **8**, 45. http://dx.doi.org/10.1186/1476-511X-8-45

(13) Plat, J., Brufau, G., Dallinga-Thie, G.M., Dasselaar, M., & Mensink, R.P. (2009) *J. Nutr.* **139**, 1143–1149. http://dx.doi.org/10.3945/jn.108.103481

(14) Clifton, P.M., Mano, M., Duchateau, G.S., van der Knaap, H.C., & Trautwein, E.A. (2008) *Eur. J. Clin. Nutr.* **62**, 968–977. http://dx.doi.org/10.1038/sj.ejcn.1602814

(15) Plana, N., Nicolle, C., Ferre, R., Camps, J., & Cos, R. (2008) *Eur. J. Nutr.* **47**, 32–39. http://dx.doi.org/10.1007/s00394-007-0693-4

(16) Judd, J.T., Baer, D.J., Chen, S.C., Clevidence, B.A., Muesing, R.A., Kramer, M., & Meijer, G.W. (2002) *Lipids* **37**, 33–42. http://dx.doi.org/10.1007/s11745-002-0861-y

(17) Davidson, M.H., Maki, K.C., Umporowicz, D.M., Ingram, K.A., Dicklin, M.R., Schaefer, E., Lane, R.W., McNamara, J.R., Ribaya-Mercado, J.D., Perrone, G., Robins, S.J., & Franke, W.C. (2001) *J. Am. Coll. Nutr.* **20**, 307–319. http://dx.doi.org/10.1080/07315724.2001.10719051

(18) Jones, P.J., Raeini-Sarjaz, M., Ntanios, F.Y., Vanstone, C.A., Feng, J.Y., & Parsons, W.E. (2000) *J. Lipid Res.* **41**, 697–705
(19) Shaghaghia, M.A., Harding, S.V., & Jones, P.J.H. (2014) *J. Funct. Foods* 6, 280–289. http://dx.doi.org/10.1016/j.jff.2013.10.017
(20) Rideout, T.C., Ramprasath, V., Griffin, J.D., Browne, R.W., Harding, S.V., & Jones, P.J. (2014) *Lipids Health Dis.* **13**, 5
(21) Ntanios, F.Y., & Jones, P.J. (1998) *Biochim. Biophys. Acta* **1390**, 237–244. http://dx.doi.org/10.1016/S0005-2760(97)00196-3
(22) Ntanios, F.Y., van de Kooij, A.J., de Deckere, E.A., Duchateau, G.S., & Trautwein, E.A. (2003) *Atherosclerosis* **169**, 41–50. http://dx.doi.org/10.1016/S0021-9150(03)00132-1
(23) Vanstone, C.A., Raeini-Sarjaz, M., & Jones, P.J. (2001) *J. Nutr. Biochem*. **12**, 565–574. http://dx.doi.org/10.1016/S0955-2863(01)00175-9
(24) Jain, D., Ebine, N., Jia, X., Kassis, A., & Marinangeli, C. (2008) *J. Nutr. Biochem.* **19**, 229–236. http://dx.doi.org/10.1016/j.jnutbio.2007.02.012
(25) Liang, Y.T., Wong, W.T., Guan, L., Tian, X.Y., Ma, K.Y., Huang, Y., & Chen, Z.Y. (2011) *Atherosclerosis* **219**, 124–133. http://dx.doi.org/10.1016/j.atherosclerosis.2011.06.004
(26) Ebine, N., Demonty, I., Jia, X., & Jones, P.J. (2006) *Int. J. Obes (London)* **30**, 751–757. http://dx.doi.org/10.1038/sj.ijo.0803191
(27) Rideout, T.C., Harding, S.V., & Jones, P.J. (2010) *Mol. Nutr. Food Res.* **54**, (Suppl 1), S7–S13. http://dx.doi.org/10.1002/mnfr.201000027
(28) Volger, O.L., van der Boom, H., de Wit, E.C., van Duyvenvoorde, W., & Hornstra, G. (2001) *Arterioscler. Thromb. Vasc. Biol.* **21**, 1046–1052. http://dx.doi.org/10.1161/01.ATV.21.6.1046
(29) Plosch, T., Kruit, J.K., Bloks, V.W., Huijkman, N.C., Havinga, R., Duchateau, G.S., Lin, Y., & Kuipers, F. (2006) *J. Nutr.* **136**, 2135–2140
(30) Brufau, G., Kuipers, F., Lin, Y., Trautwein, E.A., & Groen, A.K. (2011) *PLoS One* **6**, e2157. http://dx.doi.org/10.1371/journal.pone.0021576
(31) Looije, N.A., Risovic, V., Stewart, D.J., Debeyer, D., Kutney, J., & Wasan, K.M. (2005) *J. Pharm. Sci.* **8**, 400–408
(32) Lukic, T., Wasan, K.M., Zamfir, D., Moghadasian, M.H., & Pritchard, P.H. (2003) *Metabolism* **52**, 425–431. http://dx.doi.org/10.1053/meta.2003.50084
(33) Schonewille, M., Brufau, G., Shiri-Sverdlov, R., Groen, A.K., & Plat, J. (2014) *J. Lipid Res.* **55**, 2554–2561. http://dx.doi.org/10.1194/jlr.M052407
(34) Matsuoka, R., Muto, A., Kimura, M., Hoshina, R., Wakamatsu, T., & Masuda, T. (2008) *J. Oleo. Sci.* **57**, 309–314. http://dx.doi.org/10.5650/jos.57.309
(35) Awaisheh, S.S., Khalifeh, M.S., Al-Ruwaili, M.A., Khalil, O.M., Al-Ameri, O.H., & Al-Groom, R. (2013) *J. Dairy Sci.* **96**, 9–15. http://dx.doi.org/10.3168/jds.2012-5442
(36) Ikeda, I., Konno, R., Shimizu, T., Ide, T., & Takahashi, N. (2006) *Biochim. Biophys. Acta* **1760**, 800–807. http://dx.doi.org/10.1016/j.bbagen.2006.02.017
(37) Tomoyori, H., Kawata, Y., Higuchi, T., Ichi, I., Sato, H., Ikeda, I., & Imaizumi, K. (2004) *J. Nutr.* **134**, 1690–1696
(38) Brufau, G., Canela, M.A., & Rafecas, M. (2006) *Lipids* **41**, 159–168. http://dx.doi.org/10.1007/s11745-006-5084-8
(39) Brufau, G., Canela, M.A., & Rafecas, M. (2007) *J. Nutr. Biochem.* **18**, 580–586. http://dx.doi.org/10.1016/j.jnutbio.2006.10.003
(40) Brufau, G., Canela, M.A., Quílez, J., & Rafecas, M. (2008) *Eur. J. Lipid Sci. Tech.* **110**, 206–215. http://dx.doi.org/10.1002/ejlt.200700086
(41) Naumann, E., Plat, J., Kester, A.D., & Mensink, R.P. (2008) *J. Am. Coll. Nutr.* **27**, 117–126. http://dx.doi.org/10.1080/07315724.2008.10719683
(42) Demonty, I., Ras, R.T., van der Knaap, H.C., Meijer, L., & Zock, P.L. (2013) *Eur. J. Nutr.* **52**, 153–160. http://dx.doi.org/10.1007/s00394-011-0297-x
(43) Rideout, T.C., Harding, S.V., Mackay, D., AbuMweis, S.S., & Jones, P.J. (2010) *Am. J. Clin. Nutr.* **92**, 41–46. http://dx.doi.org/10.3945/ajcn.2009.29073
(44) Uchida, A., Slipchenko, M.N., Cheng, J.X., & Buhman, K.K. (2011) *Biochim. Biophys. Acta* **1811**, 170–176. http://dx.doi.org/10.1016/j.bbalip.2010.12.011
(45) Bunger, M., van den Bosch, H.M., van der Meijde, J., Kersten, S., Hooiveld, G.J., & Muller, M. (2007) *Physiol. Genomics* **30**, 192–204. http://dx.doi.org/10.1152/physiolgenomics.00198.2006
(46) de Vogel-van den Bosch, H.M., Bunger, M., de Groot, P.J., Bosch-Vermeulen, H., Hooiveld, G.J., & Muller, M. (2008) *BMC Genomics* **9**, 231. http://dx.doi.org/10.1186/1471-2164-9-231
(47) Gerson, T., Shorland, F.B., & Dunckley, G.G. (1965) *Biochem. J.* **96**, 399–403
(48) Konlande, J.E., & Fisher, H. (1969) *J. Nutr.* **98**, 435–442
(49) Malini, T., & Vanithakumari, G. (1990) *J. Ethnopharmacol.* **28**, 221–234. http://dx.doi.org/10.1016/0378-8741(90)90032-O
(50) Plat, J., & Mensink, R.P. (2002) *Eur. J. Clin. Invest.* **32**, 242–250. http://dx.doi.org/10.1046/j.1365-2362.2002.00982.x
(51) Rudkowska, I., AbuMweis, S.S., Nicolle, C., & Jones, P.J. (2008) *Appl. Physiol. Nutr. Metab.* **33**, 728–734. http://dx.doi.org/10.1139/H08-041
(52) Houweling, A.H., Vanstone, C.A., Trautwein, E.A., Duchateau, G.S., & Jones, P.J. (2009) *Eur. J. Clin. Nutr.* **63**, 543–551. http://dx.doi.org/10.1038/sj.ejcn.1602969
(53) Gazi, I.F., Daskalopoulou, S.S., Nair, D.R., & Mikhailidis, D.P. (2007) *Curr. Med. Res. Opin.* **23**, 2183–2192. http://dx.doi.org/10.1185/030079907X226267
(54) Geelen, A., Zock, P.L., de Vries, J.H., & Katan, M.B. (2002) *Eur. J. Clin. Invest.* **32**, 738–742. http://dx.doi.org/10.1046/j.1365-2362.2002.01061.x